Characterization and Physiological Activity of Some *Kawa* Constituents

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Kawa, THE RHIZOME OF *Piper methysticum,* has played an important role in the lives of the peoples of Oceania. This paper presents a review of our current knowledge of the *kawa* constituents—their molecular structure, their pharmacological properties, and their chemical and spectroscopic character.

AROMATIC CONSTITUENTS AND THEIR PHYSIOLOGICAL ACTIVITY

Piper methysticum Forster is a shrub in the family Piperaceae which occurs in Oceania. Its striking characteristics are long-stemmed, heart-shaped leaves, peculiarly knotty branches, and small flowers which lack a perianth and which form a dense inflorescence reminiscent of ears of grain. It is one of the bisexual members of the genus; apparently, however, only plants with male flowers exist. This necessitates vegetative propagation of the species. The original habitat of the plant is not known; perhaps it is New Guinea. From the rhizome, less often from a sprout, Polynesians and Melanesians prepare the so-called *kawa, 'awa,* or *yangona* drink, which is characterized by its peculiar sedative and muscle-relaxing effects. In order to understand the role which the *kawa* plant played in the life of these peoples one has to consider the endemic character of the flora of these regions. The flora of Oceania is markedly different from that of the Asian continent as well as from that of the American continent. Nature did not provide the Polynesians with intoxicants and stimulants of plant origin, substances of the types which were known to the peoples of Asia, Europe, and America. Most of the indispensable medicinal agents—such as opium, hashish, coca leaves, the *Solanum* drugs, digitalis, and *Colchicum*—of the peoples of the old world and of the American continent remained unknown to the Polynesians. Nor did they have alcohol. They knew only one stimulant which at the same time was an effective medicine: *'awa.* Apparently, *'awa* for the Polynesians was a stimulant, an anesthetic, and an effective medicine. A plant of such importance, of course, also played a role in the religious life: *kawa* was the drink at religious ceremonies.

If one summarizes the numerous reports in the ethnological literature about the folk medicinal uses of the drug and translates them into modern scientific language, the following striking properties of *kawa* emerge (Lewin, 1886; Titcomb, 1948; van Veen, 1938): (1) It removes tension and anxiety. (2) It is an analgesic. (3) Small doses relax the muscles of the extremities, while larger doses paralyze through relaxation, without, however, causing a decrease in consciousness or will power. (4) The reversible relaxation or paralysis of the muscles of the extremities is not as pronounced as it is with curare; it also probably acts on the central nervous system. (5) The drug is active against various skin diseases. (6) Consumption of *kawa* may lead to photophobia.

Modern research has dealt with two major tasks: first, to recognize which constituents are responsible for the effects listed here; and second, to analyze the effects themselves through studies with experimental animals. The emphasis in the following discussion will be on pharmaco-chemical considerations. Results of pharmacological investigations will be treated only parenthetically.

The characteristic constituents of kawa

If one chews a piece of *kawa,* the tip of the tongue quickly becomes numb, as if one had chewed coca leaves. All of the early investigators associated *kawa* with cocaine, the local anesthetic principle of *Folia cocae.* This association, later shown to be false, directed *kawa* research into certain channels. First, a belief persisted until very recently that *kawa* must have nitrogenous principles (alkaloids) as does coca. Every so often a report appeared which

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claimed that alkaloids were actually demonstrated (only, however, on the basis of color reactions), but actual isolation of nitrogenous compounds has never been successful. The fact that the usual testing reagents for alkaloids are relatively non-specific was not taken into account. Especially ignored was the fact that the α-pyrones (which contain no nitrogen) can behave similarly to alkaloids in some of these tests (Farnsworth et al., 1962). Furthermore, L. Lewin, a toxicologist and pharmacologist in Berlin, who studied the drug intensively from 1880 on, was able to demonstrate that the anesthetic principle could be extracted from the plant with fat solvents, such as petroleum ether. Since that time, pharmacognosists and chemists have examined almost exclusively the lipid fraction of the plant.

MOLECULAR BUILDING BLOCKS OF THE *kawa* LACTONES (PYRONES): Nine lactones and two chalcones (i.e., eleven aromatic compounds) have so far been isolated from the plant. None of these occur as glycosides. We shall first consider the lactones, all of whose structures follow the pattern shown in Figure 1. The basic skeleton consists of 13 carbon atoms, 6 of them in a benzene ring. The benzene ring is linked by a 2-carbon bridge to an unsaturated 6-membered lactone. At first sight this lactone is reminiscent of sorbic acid lactones or of the bufadienolides

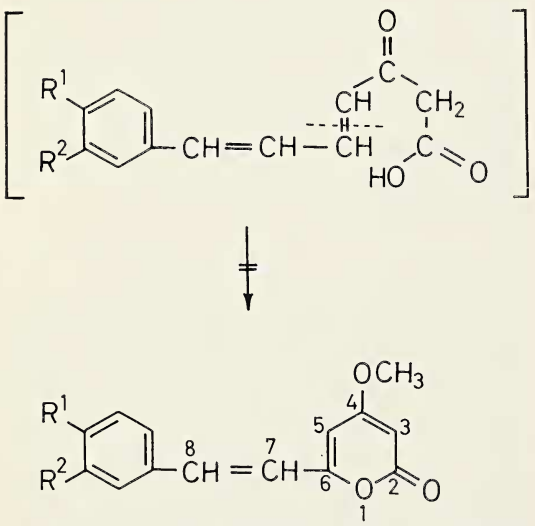


FIG. 1. Common molecular skeleton of the *kawa* lactones and their hypothetical precursor.

which are cardiac-active glycosides isolated from the plant genus *Scilla* and from toads. However, an important difference should be noted. The *kawa* lactones contain a methoxyl group as part of an enol ether function, which strongly modifies the behavior of the lactone toward acids and alkalies, as compared with the bufadienolides and other known δ-lactones. In the *kawa* lactones we are dealing with masked enols of β-diketones. The chemical behavior of the *kawa* lactones is summarized in Figure 2 using kawain and dihydrokawain as examples (Borsche et al., 1914–1933).

NATURALLY OCCURRING STRUCTURAL VARIANTS: We have seen that the characteristic constituents of *kawa* are δ-lactones, which may be considered to be α-pyrones bearing a methoxyl group at carbon 4 and an aromatic styryl or phenylethyl moiety at carbon 6. The question arises what structural variants of this basic skeleton occur in nature. We can distinguish two groups of structural variants: (a) variance depending on the degree of saturation, and (b) variance depending on benzene substitution.

(a) We note that the molecule of the basic skeleton, the styryl-α-pyrone, contains three non-aromatic double bonds. If one assumes that in the biological environment, that is, in the plant, each of these three double bonds can be saturated independently, eight variants are conceivable which would differ from one another with regard to the degree of saturation. Theoretically, there is no difficulty in synthesizing all eight variants. Nature, however, seems to follow its own laws in that not all variants which are synthetically available in the laboratory occur in the plant; a certain limitation or choice exists. In our case the following situation obtains. The *kawa* plant lacks all those variants which contain the reduced double bond at carbon 3. To put this positively, all naturally occurring *kawa* lactones invariably contain in the molecule the enolic double bond. The number of structural variants which occur in the plant is therefore reduced to 4. Another phenomenon is present. The double bond at carbon 7 is reduced only when the double bond at carbon 5 is also in the reduced state. These relationships are summarized in Figure 3.

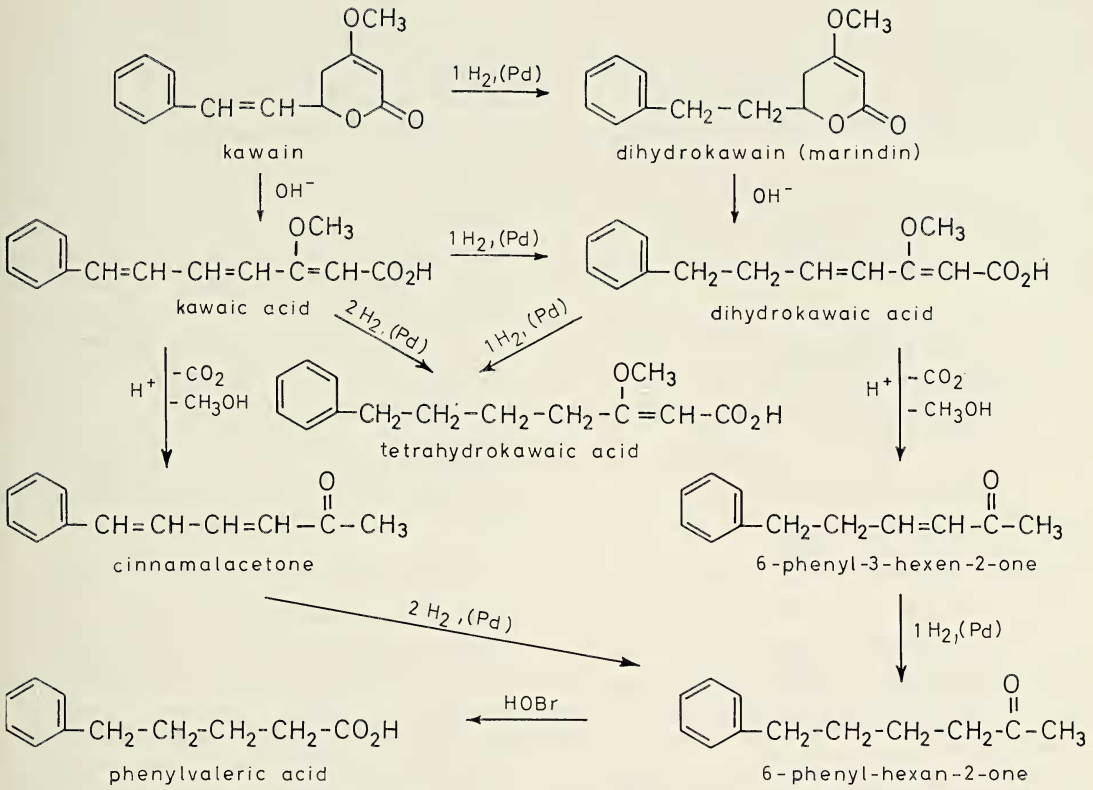
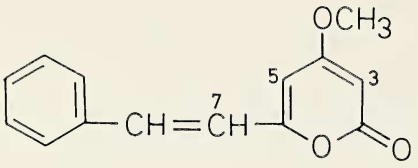


FIG. 2. Chemical reactions of kawain and methysticin (only kawain is shown).

(b) Nothing very remarkable can be said regarding the substituents on the benzene ring. In addition to unsubstituted derivatives we find the corresponding p-methoxy derivatives, dimethoxy derivatives, and dioxymethylene compounds. No lactones occur in *kawa* which have a free hydroxyl group to which sugars could be linked to form glycosides. Lactones with a free or a glycosidically linked hydroxy group therefore do not occur. Of course we have no answer to the question why the *kawa* plant does not produce free phenols or glycosides. This may have to do with the excretion cells of the plant. A particular constituent, in order to be deposited in the excretion cells of the Piperaceae, must have a certain lipid solubility, a phenomenon which is well known from the constituents of essential oils. Substances with a free hydroxy group, or even with glycosidically linked hydroxy groups, obviously do not have a suitable partition coefficient for deposition in the excretion spaces. Of course, the correlation may also be reversed: since the plant synthesizes many lipophilic end products, excretion cells are formed. Be that as it may, a correlation no doubt exists.

If we now combine the two possibilities for variation, (i) the degree of hydrogenation, that is, the number of non-aromatic double bonds, and (ii) benzene substitution, we arrive at a possible total of twelve structural variants (Table 1). Six of these have been known for a long time. We have worked out effective analytical separation and testing methods which will allow us to find additional lactones which might occur in the plant in trace amounts. We have synthesized all twelve structural variants and a few additional ones. Furthermore we have investigated *kawa* from various parts of Oceania, using samples from the island of Hawaii, the Fiji Islands, and from Samoa. As a result we now have the following picture. In addition to the six already known lactones we have demonstrated the presence of three additional derivatives so that today nine of a total of twelve pyrones are known as constituents of *Piper*



Non-aromatic double bonds: 3

Theoretically possible variants: 8

not hydrogenated: 1

monohydrogenated
(Δ_3 or Δ_5 or Δ_7): 3

dihydrogenated
($\Delta_{3.5}$ or $\Delta_{3.7}$ or $\Delta_{5.7}$): 3

fully hydrogenated: 1

Naturally occurring
(not hydrogenated, Δ_5, $\Delta_{5.7}$): 3

FIG. 3. Hydrogenation stages of the *kawa* lactones.

methysticum (Klaproth, 1966). It is interesting that in both series of the unsubstituted lactones and of the p-methyl substituted ones we find three degrees of hydrogenation. In the dioxymethylene derivatives one of the stages of hydrogenation is missing. It is the same one which, as the only representative of the series, occurs as a dimethoxy derivative. We do not know whether this vicarious occurrence of dimethoxy- and dioxymethylene, or for that matter the entire α-pyrone spectrum of the plant, is the result of evolutionary coincidence, or whether some day we shall be able to understand this development on causal biosynthetic grounds. However, let us not linger over fruitless speculations regarding the origin of the pyrone spectrum, let us rather ask in which way the plant constructs these substances. I shall not be able to offer direct proof for definite biosynthetic pathways since tracer studies or dynamic biochemical studies have so far not been carried out. The ideas of the biosynthesis of the *kawa* pyrones which I shall discuss are based on a comparative study of molecular structure and on reasoning by analogy which has derived justification from the fundamental agreement in the metabolism of all green plants.

IDEAS ON THE BIOSYNTHESIS OF THE *kawa* PYRONES: Let us consider briefly the structure of the *kawa* pyrones from a biogenetic point of view. It is striking that the benzene ring is substituted in the manner in which we know it from the phenylpropyl compounds (cinnamic acids, lignanes, and coumarins). The synthesis of these C_6–C_3-compounds goes back to shikimic and prephenic acids. The remaining four carbon atoms in the molecule of the *kawa* pyrones show two alternating oxygen functions, a feature which is characteristic of substances whose biosynthesis indicates polyacetate chains, that is, acetate metabolism. We thus arrive at the picture that the *kawa* pyrones are an example of so-called mixed formation. They are formed from phenylpropanes and from acetyl coenzyme A building blocks and, if one considers numbers, from one phenylpropane and two acetate units:

$$C_6\text{–}C_3 + 2\,C_2 \longrightarrow C_{13}\ (\textit{kawa}\ \text{pyrones})$$

Such a scheme is outlined in Figure 4. Natural products which demonstrate such a mixed construction from phenylpropanes and acetates are no rarity in the plant kingdom. The most important representatives are the flavonoids, which are made up of one phenylpropane unit and three acetate units (Geissman and Hinreiner, 1952).

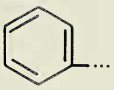
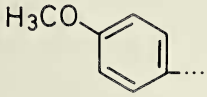
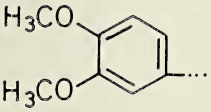
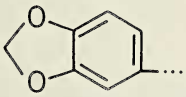
$$C_6\text{–}C_3 + 3\,C_2 \longrightarrow C_{15}\ (\text{flavonoids})$$

The *kawa* pyrones therefore appear to be nothing but variants of flavonoids. Of course this holds only if one considers the metabolic physiological and not the analytical chemical point of view, since no flavonoid-like C_{15}-compounds are known with α-pyrone structure. Flavonoids and *kawa* pyrones seem to have a common precursor. *Kawa* pyrones appear to be precursors of flavonoids with one less acetate unit. It is very remarkable that it was possible to discover in the *kawa* plant the pyrones which, so to speak, correspond to flavonoids, C_{13}-compounds which are analogs of C_{15}-compounds (Fig. 5) (Hänsel et al., 1963).

Up to this point our biosynthetic scheme assigns to the *kawa* lactones a given position in the flavonoid metabolism of plants. It does not explain why only certain degrees of hydrogenation occur in nature. The following biosynthetic scheme combines the observation on the occurrence of certain hydrogenation types and places

TABLE 1

STRUCTURAL VARIANTS OF THE KAWA LACTONES AND THEIR OCCURRENCE IN NATURE[1]

Benzene-substitution	Hydrogenation Δ5	Hydrogenation Δ7	Name
	–	–	demethoxyyangonin
	+	–	kawain
	+	+	dihydrokawain
	–	–	yangonin
	+	–	Δ5-dihydroyangonin*)
	+	+	tetrahydroyangonin*)
	–	–	11-methoxyyangonin*)
	+	–	– – –
	+	+	– – –
	–	–	– – –
	+	–	methysticin
	+	+	dihydromethysticin

[1] Symbols used: * Occurs in trace amounts in *Piper methysticum*.
– – – Does not occur in *Piper methysticum*.

it into a hypothetical biosynthetic scheme. In this scheme (Fig. 6) the paths which lead on one hand to kawain and on the other hand to yangonin branch already at the stage of the β, δ-diketocarboxylic acid. Reduction of the δ-keto group to an alcohol leads after cyclization to the enolic kawain and dihydrokawain. Enolization of the δ-keto group, however, followed by cyclization leads to the dienolic yangonin. In order to arrive at the hypothetical (synthetically easily accessible, but not occurring in nature) 7,8-dihydroyangonin derivatives, the long conjugated system of yangonin would have to be interrupted. One therefore suspects that hydrogenation of the double bond at this point is not possible for energetic reasons. In the case of

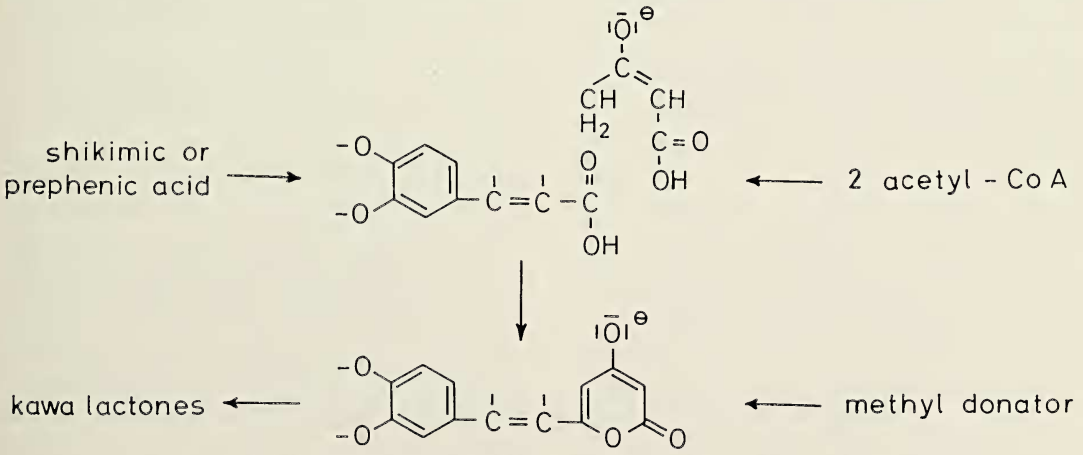


FIG. 4. A hypothetical scheme for the biosynthesis of the *kawa* lactones.

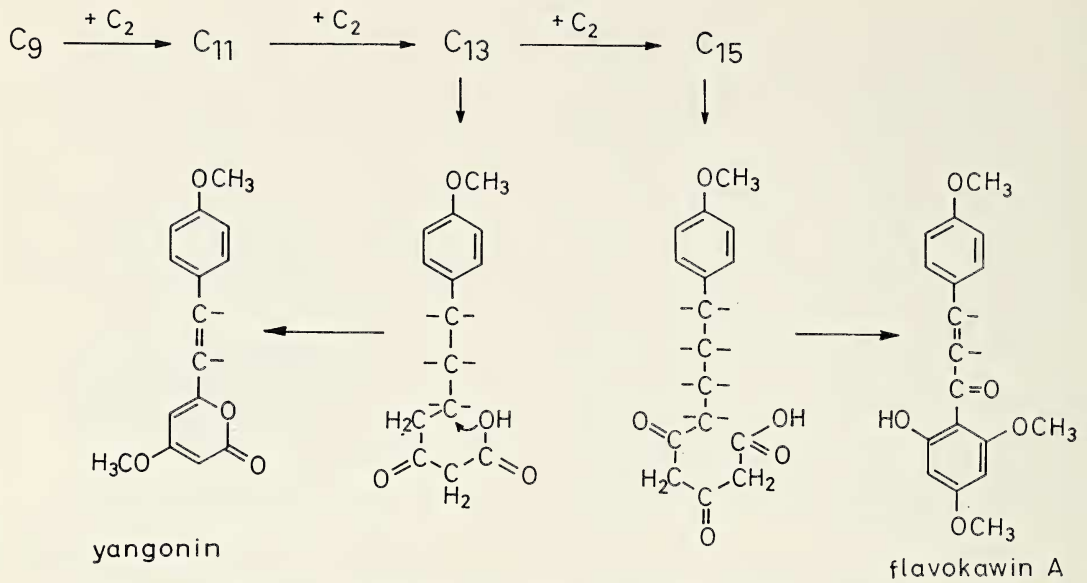


FIG. 5. Biosynthetic scheme for the formation of yangonin and a C-15 chalcone from a common precursor.

kawain, no long conjugated system is interrupted when the 7,8-double bond is reduced. The dihydrokawain derivatives indeed occur in nature.

Physiological Activity of Kawa *Pyrones*

The most intensive study of the pharmacology of the *kawa* pyrones has been carried out for some ten years by a team at the Pharmacological Institute of the University of Freiburg under the direction of H. J. Meyer. The most important observations on the effects of the *kawa* pyrones seem to be the following: intensification of barbiturate narcosis (Klohs et. al., 1959; Meyer, 1962); analgetic effect (Brüggemann and Meyer, 1963); local anesthetic properties (Meyer and May, 1964); anticonvulsive effects (Meyer, 1964; Meyer and Meyer-burg, 1964; Kretzschmar and Meyer, 1965); spasmolytic effect (Meyer, 1965*a*); antimycotic effects (Hänsel, Weiss, and Schmidt, 1966).

I should like to make another general observation on the relationship between pharmacodynamic activity of the different *kawa* pyrones and their constitution. As far as animal experiments are concerned the lactones of the yangonin type, that is the dienolides, seem to be—within the usual dosages and as compared with enolides—pharmacodynamically inert.

In the enolides the effective optimum varies as a function of the hydrogenation of the double-bonded carbon 7 and of substitution in the benzene ring together with a dependence on the method of testing. For example kawain has the strongest effect as a local anesthetic, dihydromethysticin as a spasmolytic, and dihydrokawain as an intensifier of narcosis.

INTENSIFICATION OF BARBITURATE NARCOSIS (Klohs et al., 1959; Meyer, 1962): When a pharmacologist has the task of testing pharmacologically little investigated substances for central sedative properties, he will probably measure first the toxicity of the substance and then the decrease in spontaneous motility. At a very early stage of the screening process he will check whether and in which way his substances will influence the effect of barbiturates. Substances with a central paralyzing effect intensify the effect of barbiturates and/or considerably prolong the effect. Dihydromethysticin (DHM) possesses to a particularly high degree this effect of intensifying barbiturate effects (intensifying narcosis). Let me cite an example (Meyer, 1962). After application of 150 mg/kg of hexobarbital sodium, white mice sleep on the average for 2 hours. If the animals are pretreated with 240 mg/kg DHM, they sleep after the

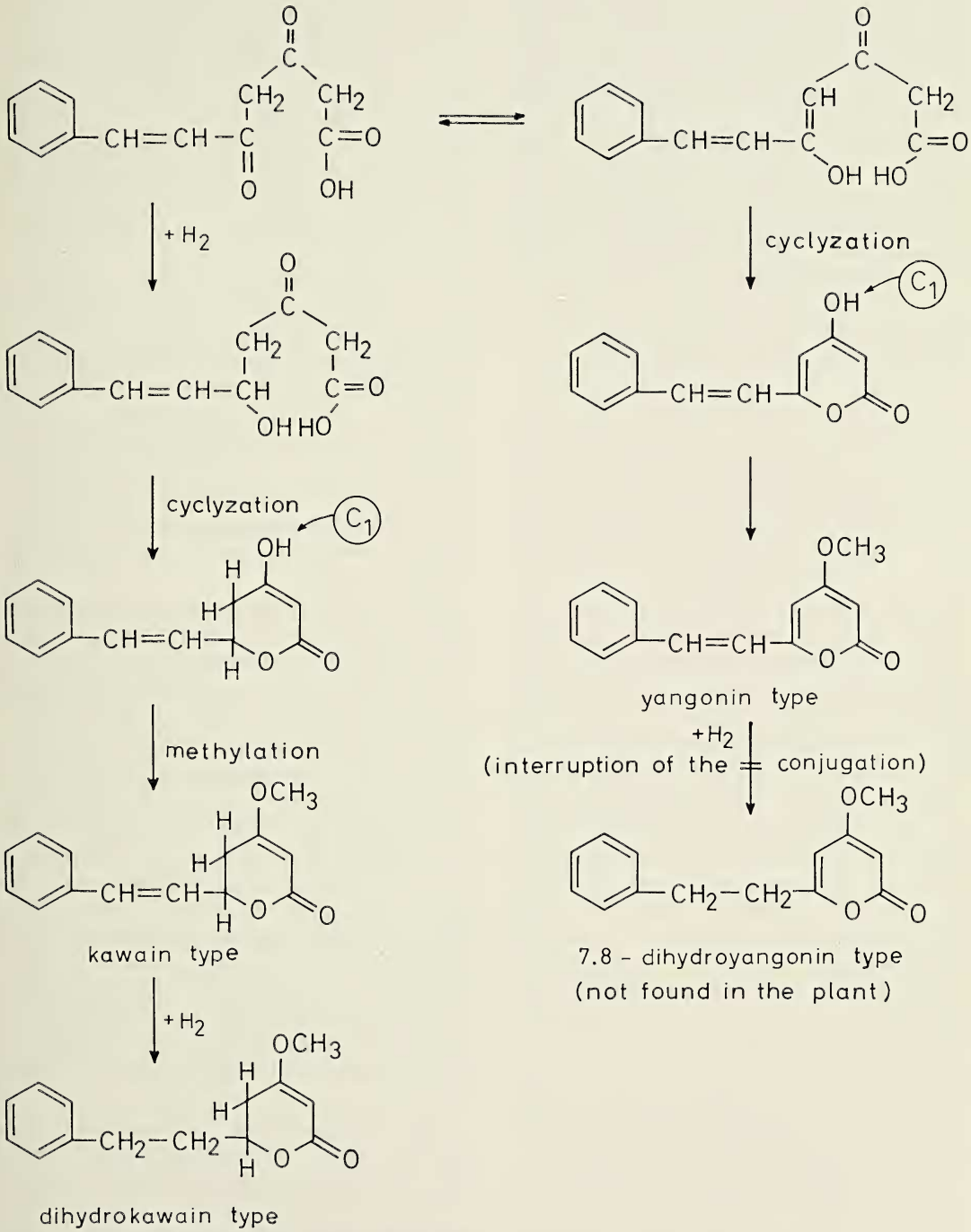


FIG. 6. Biosynthetic scheme showing various stages of hydrogenation.

same dose of hexobarbital sodium for 27 hours. Enhancement of barbiturate narcosis is a property which is shared by a relatively large number of substances with central sedative properties. What is very impressive in the case of DHM, however, is the magnitude of the effect.

ANALGETIC EFFECT: Together with Brüggemann, a team under K. H. Meyer in the Pharmacological Institute of the University of Freiburg tested the analgetic effectiveness of the two *kawa* pyrones, DHK (dihydrokawain) and DHM. In the analgesic test according to Gross these two substances prove to be comparable in effect with dimethylaminophenazone. These results are summarized in Table 2.

LOCAL ANESTHETIC PROPERTIES: I have mentioned earlier that chewing crude *kawa* anesthetizes the tip of the tongue. This anesthetic effect is caused by DHK, a fact which has been known for a long time (van Veen, 1938). Again it was H. J. Meyer and his collaborators who carried out the detailed study of the local anesthetic properties using the testing methods of scientific pharmacology. It was shown, first, that not only DHK but that all the other *kawa* pyrones possess local anesthetic properties; however, not all pyrones have an equally strong effect. It was further demonstrated that the pyrones developed superficial anesthetic as well as infiltration effects. I shall mention a few details of the experiment on surface anesthesia (rabbit cornea) and the results. Most effective with respect to the degree of hydrogenation are the $\triangle^{5}$-dihydro derivatives, followed by the $\triangle^{5,7}$-tetrahydro derivatives; the non-hydrogenated yangonin homologues are ineffective. Unsubstituted derivatives are more effective than dioxymethylene substituted ones. Accordingly, the most effective compound in surface anesthesia is kawain which equals the effect of cocaine in the cornea test. Kawain and cocaine possess the same limiting concentration (equal to the smallest concentration which causes complete anesthesia in all animals) and equal length of anesthesia. The *kawa* pyrones are somewhat less effective in infiltration anesthesia. Of some interest for their evaluation is the fact that the *kawa* pyrones are very compatible with tissues and no danger of toxic resorption exists.

TABLE 2

RELATIVE ANALGETIC EFFECT OF DHM AND DHK
(Condensed from Brüggemann and Meyer, 1963)

ANALGETIC AGENT	DOSE (mg/kg)
morphine	2.5
dimethylaminophenazone	100
dihydrokawain	120
dihydromethysticin	140
acetylsalicylic acid (aspirin)	200

ANTICONVULSIVE EFFECTS: A cursory comparison of the total effect of *kawa* on man and of the pharmacological activity of the pyrones in the animal shows that *kawa* acts predominantly by central paralysis. In order to characterize in greater detail substances having a central paralytic effect, it is important to know toward which central nervous system (CNS) stimulants these substances act as antagonists. Furthermore, one needs to know whether they are capable of inhibiting tonic or clonic spasm components of electric shock.

Concerning the inhibition of electric shock, a whole series of well known and excellent drugs have been introduced into therapy and are frequently used as sedatives which inhibit at a given dose but which are toxic. A well known example of the statement that CNS sedatives are not necessarily effective inhibitors of electric stimulation is meprobamate. Other sedatives enhance, contrary to expectation, even the readiness for contraction; an example is reserpine. According to investigations, which again were carried out by the Freiburg team, DHM and DHK are contraction inhibitors. This inhibitory effect is qualitatively and quantitatively comparable with that of phenobarbital, pyrimidone, or diphenylhydantoin. Animal experiments have shown that DHM in particular may be considered a strongly effective anticonvulsant.

The behavior of the two *kawa* pyrones is peculiar toward chemical convulsive toxins, toward bemegride, pentetrazole, picrotoxin, and strychnine. Figure 7 shows the following: (i) Tonic bemegride and pentetrazole convulsions are activated within a given dose range (preadministration of 20-45 mg/kg DHM). (ii) Tonic picrotoxin and strychnine convulsions are

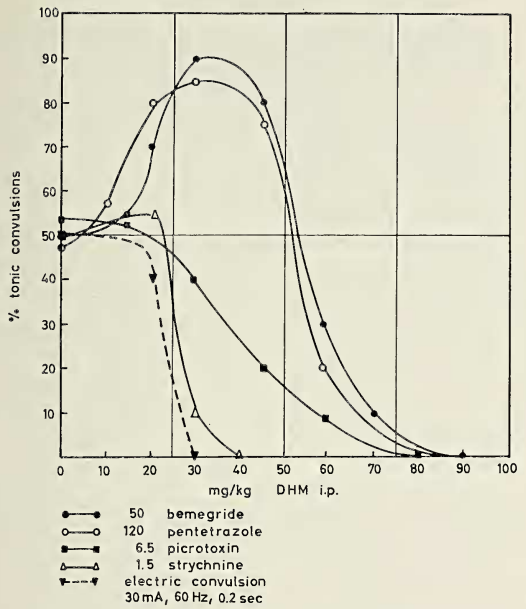


FIG. 7. Anticonvulsive activity of DHM; convulsions produced experimentally by various agents (Meyer, 1964).

inhibited by preadministration of DHM, which is similar to electric convulsions. Strychnine convulsions are inhibited more strongly than are picrotoxin convulsions.

The curves would look very different if the inhibition of *clonic* convulsions had taken place instead of *tonic* convulsions. Toward clonic convulsions, which appear after application of the above-named convulsive toxins (bemegride, pentetrazole and picrotoxin) preadministration of DHM has essentially no effect. On the contrary, DHM enhances and prolongs strikingly the clonic convulsion phase. Especially by administration of DHM one effects long lasting strong convulsions with only rare and short-lived convulsion pauses (without DHM the convulsions show a more seizure-like character). The two *kawa* pyrones DHM and DHK (the latter has a weaker effect) are therefore capable of suppressing only incompletely the convulsions caused by chemical convulsive toxins since they can suppress only the tonic but not the clonic component. A remarkable exception is strychnine. In this case DHM and DHK are able to suppress both types of convulsion. It may be said that both pyrones demonstrate a definite anti-strychnine effect, which even surpasses the well known antagonism of phenobarbital toward strychnine.

If in conclusion we ask to which long-used therapeutics the *kawa* pyrones show the greatest similarity, we may quote H. J. Meyer who, in considering recent results which are not reproduced here, says, "Aside from certain peculiarities of their action the *kawa* pyrones show the characteristics of two anticonvulsants which have long maintained a leading role in epilepsy therapy, diphenylhydantoin and phenobarbital. The similarity with the action of diphenylhydantoin is somewhat more pronounced."

SPASMOLYTIC EFFECT: Dihydromethysticin was tested thoroughly for its spasmolytic action. In all experimental designs with smooth muscle organs and with organ systems, it proved more or less effective. An inhibition of spontaneous activity as well as a relaxing effect on muscle tone could be demonstrated. The mechanism of the action was predominantly designated as musculotropic (similar to papaverine).

ANTIMYCOTIC EFFECT: Anyone who has worked in a laboratory with aqueous plant extracts has observed that, if these aqueous plant extracts stand around for some time, they spoil. The extracts become inhabited by microorganisms. We observed some time ago that aqueous extracts of *kawa* do *not* spoil, at least not while they contain traces of *kawa* pyrones. In collaboration with the Institute of Hygiene and Bacteriology of Freie Universität Berlin we have studied the bacteriostatic properties of the *kawa* pyrones. The data (Table 3) are not complete since the investigations have not terminated. I shall mention the following preliminary results (Hänsel, Weiss, and Schmidt, 1966).

(i) The *kawa* pyrones do *not* act as bacteriostats. A large number of gram positive, gram negative, pathogenic, and apathogenic bacteria were tested and they developed uninhibited in nutrients containing pyrones.

(ii) On the other hand, certain *kawa* pyrones show remarkable fungistatic effects. It is well known that there exists a large number of bacteriostats but only a very small number of substances which are capable of inhibiting the growth of fungi. Among the fungi which show a high sensitivity particularly toward DHK are

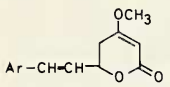
such rugged types as *Aspergillus niger*. Perhaps the best known antibiotic which is effective against fungi is griseofulvin and it has absolutely no effect on *Aspergillus niger*.

(iii) The effect of the pyrones is very selective. Only certain genera of fungi, often only certain species, are attacked. Among the fungi which are completely untouched are the yeasts —pathogenic forms as well as the ancient wine and beer yeasts. Further investigations are designed to show whether among the fungi which are affected by the *kawa* pyrones are genera which are pathogens in man.

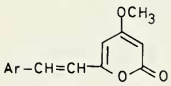
CHEMICAL AND PHYSICAL PROPERTIES OF THE *Kawa* LACTONES

Chemical Behavior

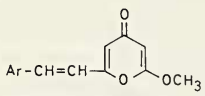
The characteristic constituents of *kawa* may be classified into two main groups. The first group, of which kawain is an example, is characterized by a single double bond in a 6-membered lactone ring. The second type, of which yangonin is an example, belongs to the series of dienolides. However both types may be considered either 6-membered lactones or 6-styryl-α-py-



kawain type



yangonin type
(dienolide, Bu'lock & Smith, 1960; Chmielewska et al. 1958)



yangonin type
(old γ-pyrone formula, Borsche, 1914)

rones. The recognition that yangonin also possesses lactone character is however rather recent (Chmielewska et al., 1958). Earlier it was considered a γ-pyrone and accordingly two series of characteristic *kawa* constituents were differentiated, the α-pyrones (lactones) and the γ-pyrones. It is obvious that the structural elucidation of yangonin provided some difficulties. In order to demonstrate these peculiar difficulties it is more useful to outline a synthesis of yangonin starting from the dimethyl derivative (yangonalactone) rather than to trace the historical development which eventually led to the

TABLE 3

INHIBITION OF SOME FUNGI AND *Streptomyces* ORGANISMS BY POWDERED KAWA RHIZOMES[1]

TEST ORGANISMS	g/ml
Trichothecium roseum	0.05
Alternaria consortiale	0.03
Penicillium funiculosum (potential human pathogen)	0.02
Alternaria humicola	0.008
Streptomyces griseus	0.005
Crytococcus neoformans (human pathogen)	0.005
Trichophyton tonsurans (human pathogen)	0.005
Streptomyces purpurescens	0.005
Paecilomyces varioti	0.0035
Aspergillus niger (potential human pathogen)	0.003
Chaetomium globosum	0.003
Trichophyton ferrugineum (human pathogen)	0.001
Botrytis cinerea	(+)
Scopulariopsis brevicaulis (potential human pathogen)	(+)
Saccharomyces cerevisiae	(+)
Aspergillus tamarii	(+)
Aspergillus flavus	(+)
Fusarium solani	(−)
Candida albicans (human pathogen)	(−)
Candida krusei	(−)
Candida parakrusei	(−)
Candida parapsilosis	(−)
Candida tropicalis	(−)

[1] The numbers are the minimum concentrations (g) of kawain per volume (ml) nutrient which completely inhibit macroscopically visible development of the fungus. (+) Indicates only growth inhibition at a concentration of 0.05 g/ml. (—) Indicates no effect at a concentration of 0.05 g/ml. (Hänsel et al., 1966).

correct formulation of yangonin. Methylation of the styryl substituted with triacetolactone (yangonalactone) with diazomethane leads to two isomeric methyl derivatives, one of which is identical with the natural product. The question remains which of the two isomeric methyl triacetolactones is identical with the natural product, the α- or the γ-pyrone. Borsche (1914)

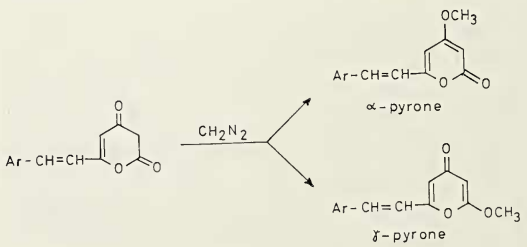


TABLE 4

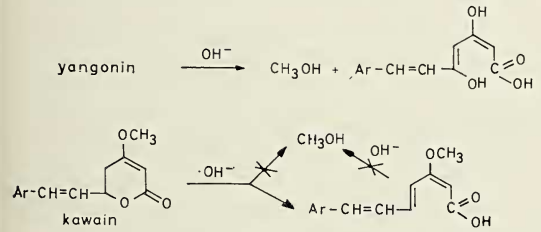
SOME DIAGNOSTIC TESTS TO DISTINGUISH BETWEEN TAUTOMERIC α- AND γ-HYDROXYPYRONE METHYL ETHERS[1]

TEST	4-METHOXY-γ-PYRONE	2-METHOXY-γ-PYRONE
Basicity		
(a) ether-insoluble oxonium salt formation	−	+
(b) picrate formation	−	+
IR carbonyl frequency		
(a) 1724 cm^{-1} (α-pyrone)	+	−
(b) 1667 cm^{-1} (γ-pyrone)	−	+
Diels-Alder reaction[2]	+	−

[1] Chmielewska et al., 1958; Bullock and Smith, 1960.
[2] Alder and Rickert, 1937.

chose a γ-pyrone formulation for the following reasons.

By the action of alkali under mild conditions yangonin can be saponified to an acid and methanol, analogous to a methyl ester of a carboxylic acid. Kawain, on the other hand, the constitution of which was secured as an α-pyrone, behaved differently. Under analogous



conditions kawain furnished no methanol, nor did kawaic acid which was produced by action of alkali. Borsche attempted to explain this striking difference in the behavior of kawain and yangonin in the following manner. Since the enol-ether linkage of kawain is not saponifiable, yangonin, which splits off methanol readily, cannot contain an enol-ether linkage. He therefore searched for a formula of yangonin which would combine an ester-like bound methanol with a cyclic structure (differing by one mole of water from an open chain ester). Borsche formulated yangonin as a γ-pyrone, which he also considered to be the "anhydride of the methanol ester of yangona acid." Borsche's ideas are summarized in Figure 8. If we consider Borsche's yangonin formula in some detail, we are rather surprised that the following points did not concern him.

(a) Ring opening with alkali is generally effected more easily with α-pyrones than with γ-pyrones.

(b) Borsche's yangonin formula has the structural characteristic of an enol acetal. Acetals are generally more resistant to alkali than are esters and vinylogous esters (enol ethers). It appears to me that it would have been possible to arrive at the opposite conclusion that the alkali liability of the ring demands formulation of an α-pyrone.

In 1954 a Polish team reopened the question of the structure of yangonin. They systematically synthesized α-pyrones and the analogous γ-pyrones and investigated both types of compounds with IR and UV spectroscopy. IR spectroscopy in particular proved to be an excellent aid for distinguishing between the two isomeric pyrones. All γ-pyrones showed a carbonyl band at 6.0μ (1667 cm^{-1}), while the α-pyrone band appears at 5.8μ (1724 cm^{-1}), which is the characteristic band of unsaturated lactones.

These investigations made it apparent that the pyrones which occur in the *kawa* rhizome differ from one another by their substituents and by their degree of hydrogenation, and not,

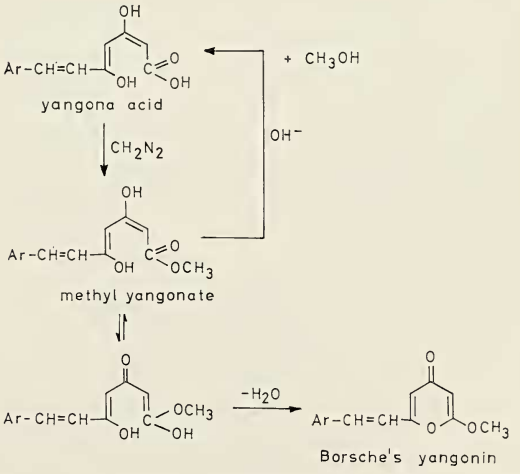


FIG. 8. Borsche's argument for the structure of yangonin.

as was originally assumed by Borsche, by being cyclic isomers (α- and γ-pyrones). If the new yangonin formula is correct, it should be possible by hydrogenation of the two double bonds to transform yangonin derivatives into dihydrokawain derivatives. We (Werny and Hänsel, 1963) were able to demonstrate that this is indeed possible. Starting with naturally occurring yangonin we obtained by catalytic hydrogenation p-methoxydihydrokawain, the constitution of which was secured by an independent synthesis. These reactions are shown in Figure 9. One of the two double bonds in the ring is not attacked under the given conditions (palladium on carbon in ethyl acetate). This agrees with the observation that enol-ether double bonds are catalytically hydrogenated only with difficulty.

The actual cause, that is the mechanism, of the phenomenon which led Borsche to the wrong formulation of yangonin is still not known. Why is the enol-methyl ether of yangonin readily

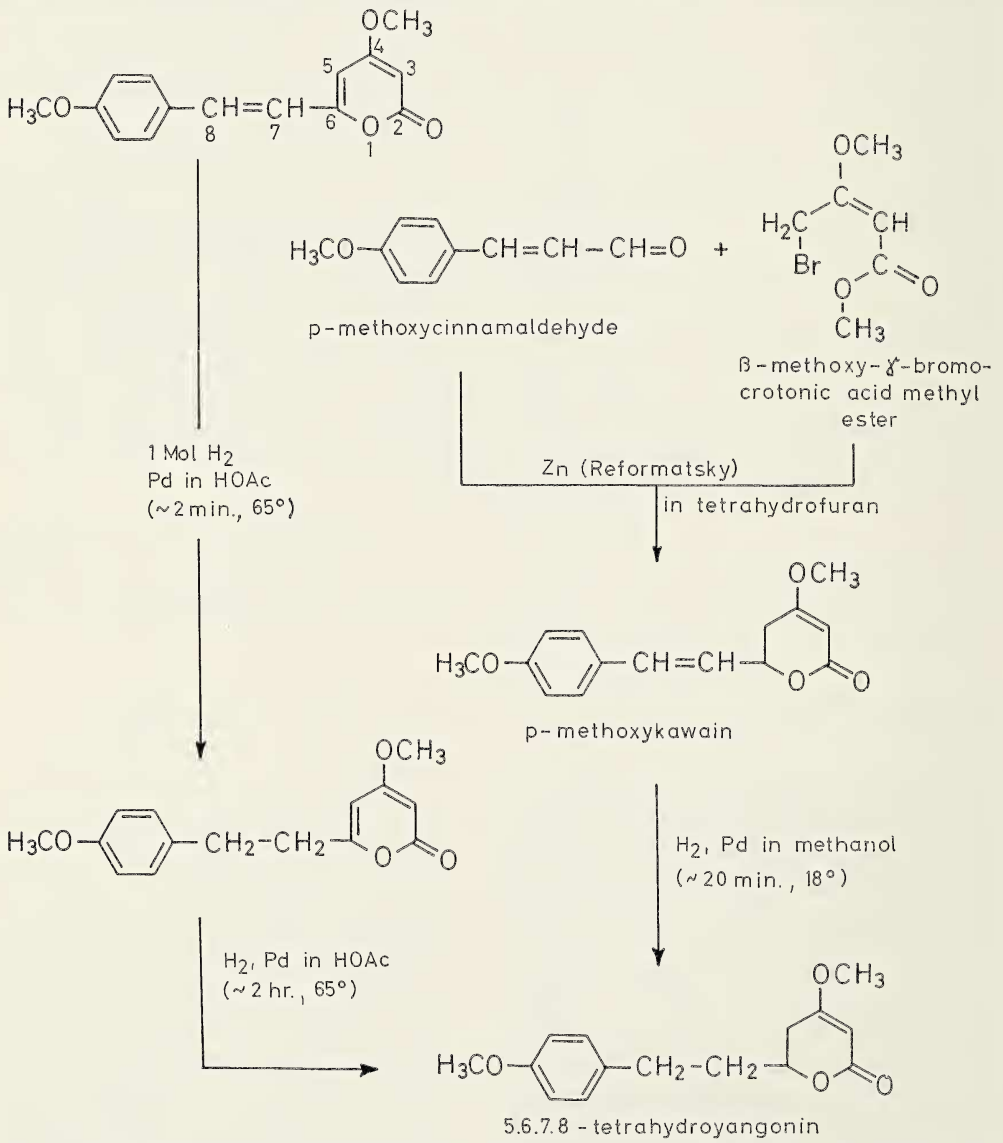


FIG. 9. Transformation of yangonin in 5.6.7.8-tetrahydroyangonin and its identity with synthetic (±) p-methoxy-7.8-dihydrokawain (Werny and Hänsel, 1963).

saponified and methanol split off only with difficulty in the case of kawain? We may reduce this question to that of the alkali stability of the enol ethers of the two homologous acids (see formulas).

$$C_6H_5—CH = CH—C(R) = CH—C(OCH_3) = CH—CO_2H$$

$$R = H \text{ (kawaic acid)} \xrightarrow{OH^-} \text{no methanol}$$

$$R = OH \text{ (yangona acid)} \xrightarrow{OH^-} \text{methanol}$$

Spectroscopic Characterization of the Kawa *Lactones*

So far in this paper we have seen that the characteristic constituents of *kawa* are substituted 6-membered lactones which may be classified into two main groups, with one or two double bonds in the ring, the enolides and the dienolides. We have seen further that these two main types may be distinguished by their behavior during alkaline hydrolysis. In the following section we will show that recognition of the hydrogenation type is achieved more quickly and more smoothly with the modern methods of IR, UV, and mass spectrometry.

IR SPECTRA (Hänsel, Rimpler, and Langhammer, 1966): It is best to start with two simple model compounds, the methyl-triacetyl acid lactone and the dihydromethyltriacetyl acid lactone (DH-MTL), which we prepared for the first time. The IR spectra of these two model compounds are exceedingly clear in the region of carbon-carbon double bond frequencies. DH-MTL exhibits in this region a single strong band at 1622 cm^{-1} which accordingly has to be assigned to the enolic double bond at $\triangle^3$. In MTL this band—probably because of conjugation of the two carbon-carbon double bonds—is shifted toward longer wave numbers by 26 cm^{-1}. We therefore assign the band at 1566 cm^{-1} to the double bond at $\triangle^5$. The assignment of one of the two hydrogenation types is also possible when the lactone rings are substituted by styryl or phenylethyl radicals. The band at 955–966 cm^{-1}, which shows a *trans* CHR = CHR linkage, is well suited for the determination whether we are dealing with a styryl or a phenylethyl type. We dealt with a total of four hydrogenation types for which we were able to develop a simple infrared assignment scheme as shown in Figure 10. The relationships are simplified and numerous details are omitted.

MASS SPECTRA OF THE *kawa* LACTONES (Pailer et al., 1965): Compounds of the yangonin type (Fig. 11) are best discussed first. We are dealing here with a conjugated system. There is no point in the molecule which facilitates the formation of energetically favorable fragments. We therefore find in this type of compound large molecular ions and little fragmentation. Repeated elimination of 28 m/e corresponds to two carbon monoxide molecules, which has been observed with coumarins. This is followed by elimination of a methoxyl group (m/e 157, 129). If we now proceed to the compounds of the kawain type (Figs. 12, 13), we notice the appearance of a peak which may be considered an elimination of cinnamaldehyde. In addition there is a peak which corresponds to the rest of the molecule which remains after cleavage of cinnamaldehyde (M — 132) = 98. In this case cinnamaldehyde represents only a small fragment while the fragment corresponding to aldehyde minus hydrogen is larger. This however is not true with the substituted derivatives. In these cases the aldehyde peak is of considerable magnitude and no carbon monoxide elimination takes place, which is observable in the unsubstituted derivative. Furthermore, the intensity of various peaks is somewhat dependent on the substitution pattern of the aromatic part of the molecule. The most striking peak in the spectrum of the compounds of the kawain type is the peak which corresponds to a benzyl or a tropylium ion. This means that quite unexpectedly cleavage takes place at a double bond. This is supposedly an artifact since presumably migration of the double bond precedes cleavage. Nevertheless, one can consider the possibility of a formal cleavage at positions with double bonds in the interpretation of the mass spectra. With substitution in the benzene ring the fragment is correspondingly displaced, that is, it is an indicator for the correct interpretation.

Finally, there remains the interpretation of substances belonging to the dihydrokawain type. It proceeds normally with formation of a tropylium ion. Cleavage p is the normal reaction of saturated lactones.

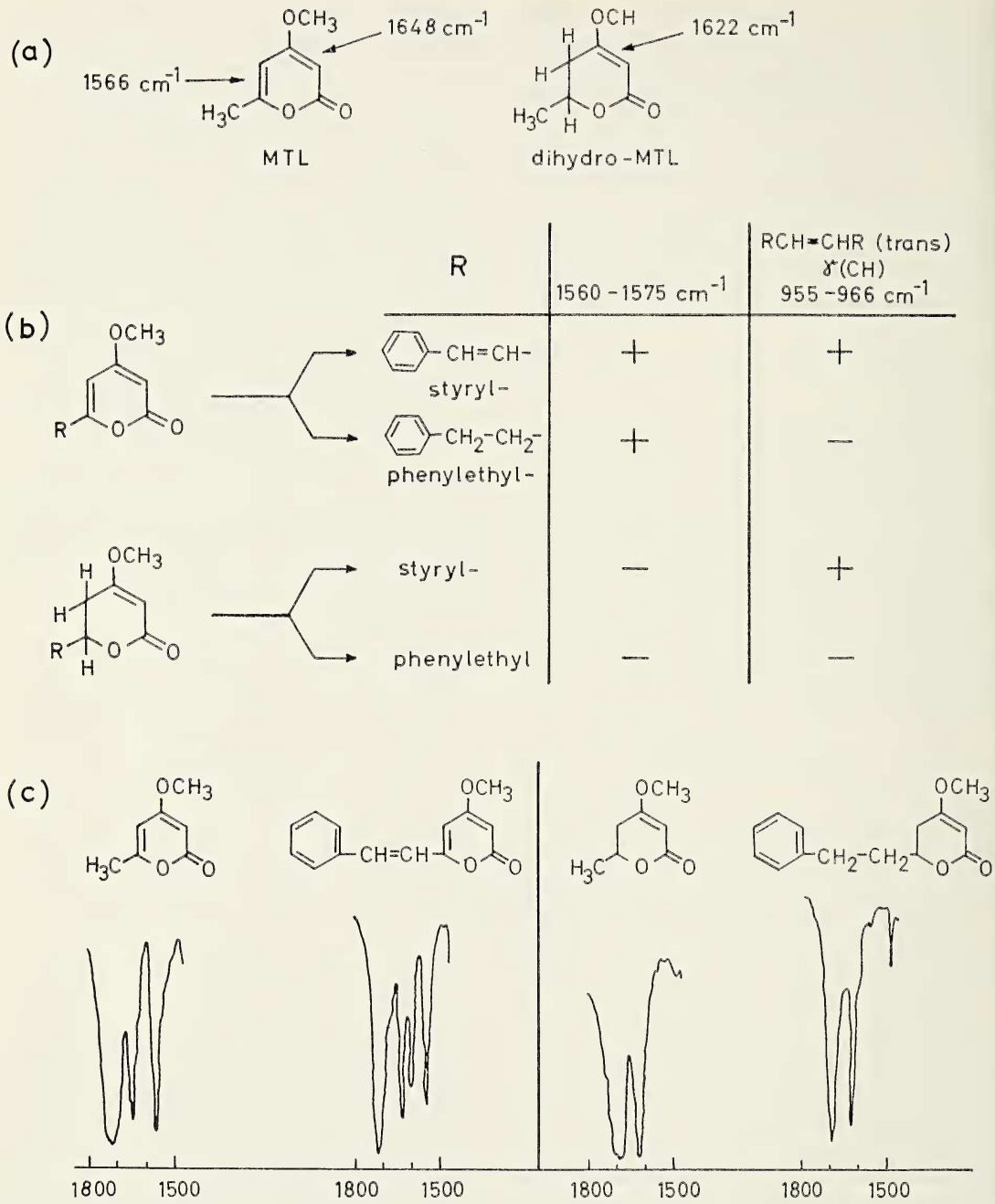


FIG. 10. Infrared spectra of the *kawa* lactones. (*a*) Assignments of cyclic double bonds; (*b*) influence of side chain; (*c*) partial spectra.

UV SPECTRA (Hänsel et al., 1967): Again it is best to begin with the basic lactone chromophore, the dihydromethyltriacetoacid lactone (DH-MTL). We are dealing here with an s-*trans*-fixed enone system, comparable to that of parasorbic acid (PSS). DH-MTL differs from PSS in the position of the maximum by an increment of 30mμ, an effect which can be ascribed to the β-methoxy substituent (Fig. 14). The magnitude of the increment is in good

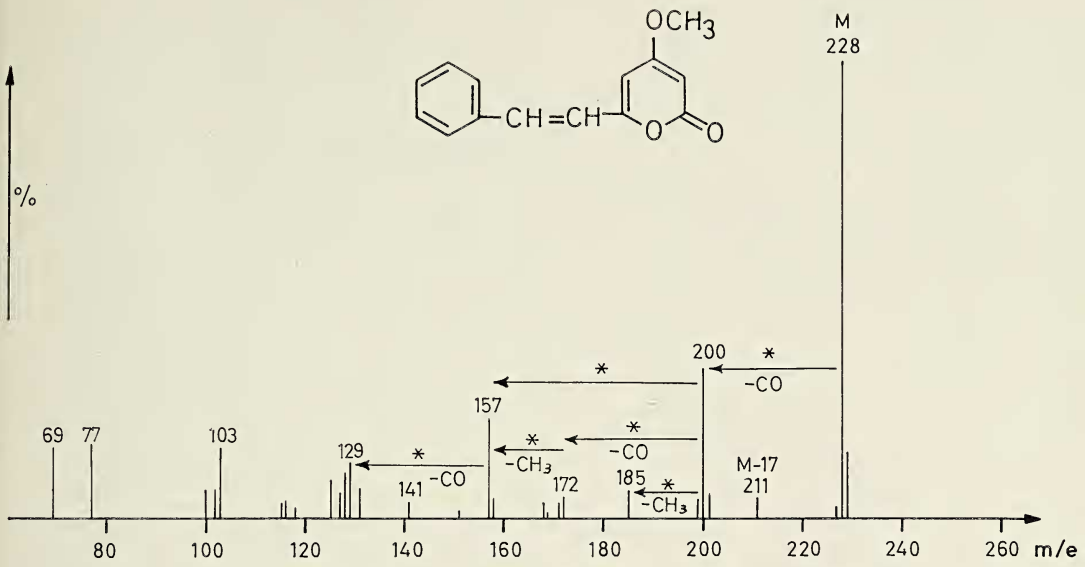


FIG. 11. Mass spectrum of dimethoxyyangonin (Pailer et al., 1965).

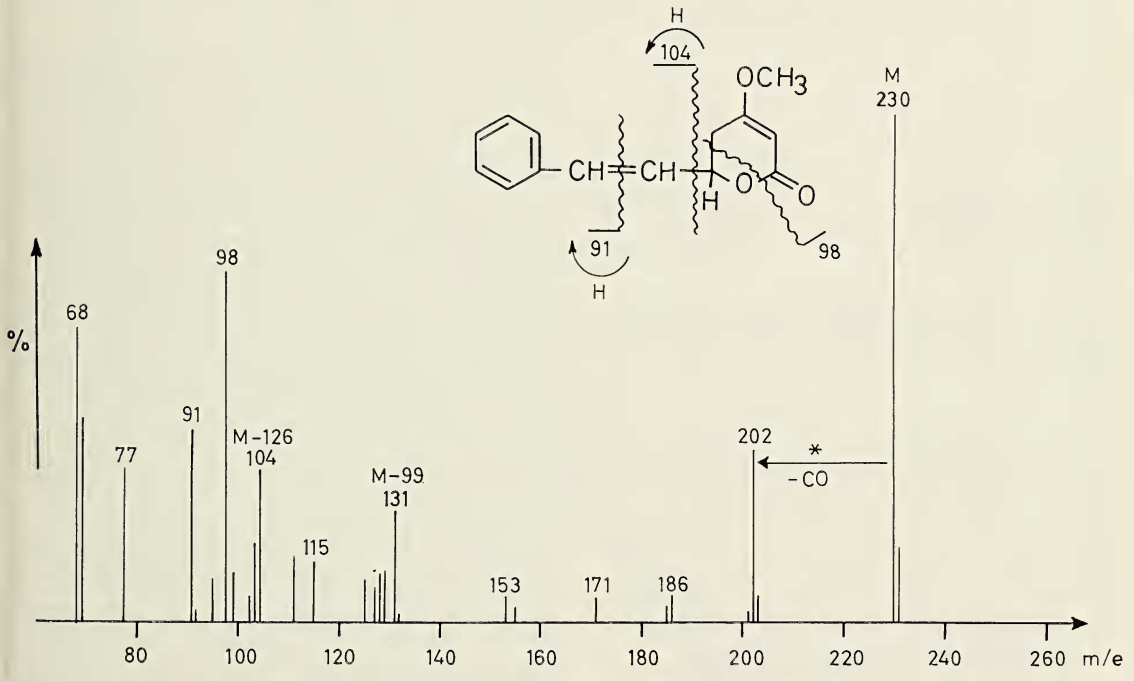


FIG. 12. Mass spectrum of kawain (Pailer et al., 1965).

agreement with observations on other open chain enone systems. Since the enone system exists in the ring, where it is rigidly maintained in the energetically favorable s-*trans* conformation, the position and magnitude of the absorption change only insignificantly when we go to the corresponding open chain enone system. In other words, we can compare PSS with *cis*-crotonic acid and dihydromethysticin (DHM) with tetrahydromethystinic acid (Fig. 15).

If we substitute the ring of DH-MTL with a phenylethyl or with a styryl radical instead of

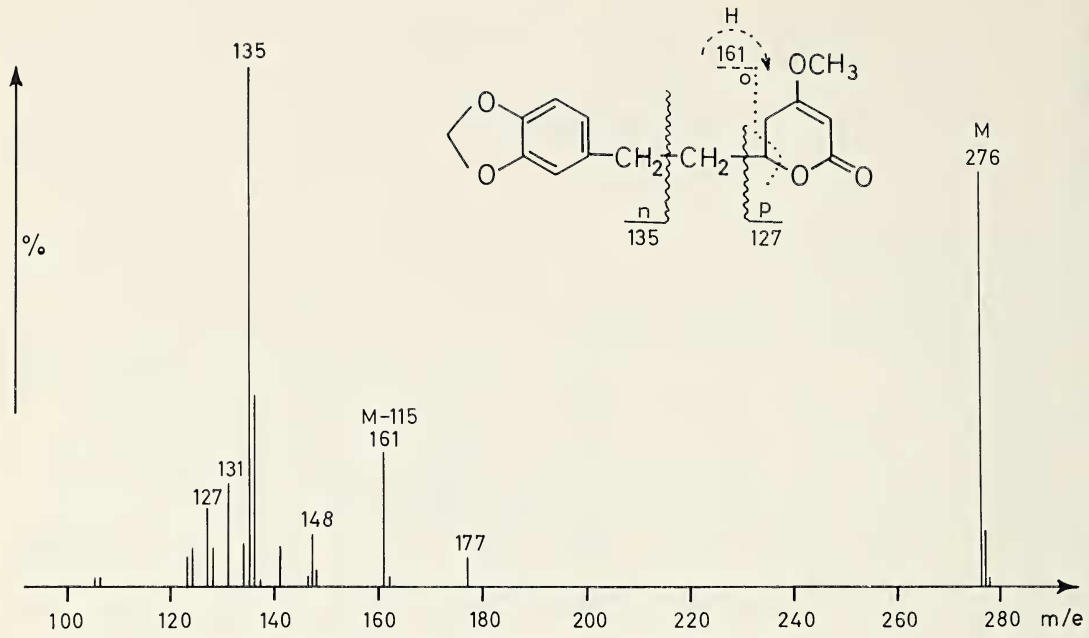


FIG. 13. Mass spectrum of dihydromethysticin (Pailer et al., 1965).

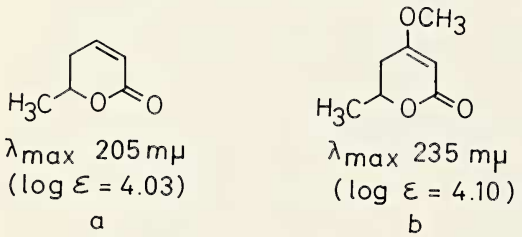


FIG. 14. UV absorption of parasorbic acid (*a*) and of dihydro-methyltriacetic acid lactone (DM-MTL) (*b*).

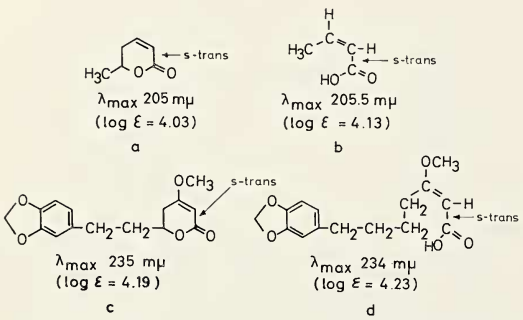


FIG. 15. UV absorption of parasorbic acid (*a*), *cis* crotonic acid (*b*), dihydromethysticin (*c*), and tetrahydromethysticinic acid (*d*).

a methyl group, we obtain two groups of natural *kawa* pyrones, the kawain and the dihydrokawain types. In the naturally occurring dihydrokawains, the two part chromophores—the benzene ring with its variable oxygen functions and the DH-MTL moiety—are separated. The spectra of these substances may therefore be interpreted as addition spectra of the two partial chromophores. This is graphically demonstrated in Figure 16 for the spectrum of tetrahydroyangonin (p-cresol methyl ether + DH-MTL). In contrast to the compounds of the dihydrokawain type we may consider that in the naturally occurring kawains the two part chromophores (styryl and DH-MTL moiety) have a mutual effect on each other. In spite of this it is surprising that the spectra of this class of compounds are also additive from given partial chromophores, that is, they may be predicted by calculation from the corresponding cinnamyl alcohols and the DH-MTL. On the other hand, measured and calculated spectra do not agree when, instead of the cinnamyl alcohols, the aromatic chromophore is represented by other styryl derivatives, such as allyphenols of the anethole type.

A second large group of *kawa* lactones are not derived from DH-MTL but from MTL itself. The two parent substances differ from each other by a double bond in the lactone ring. The enone system is extended to a dienone

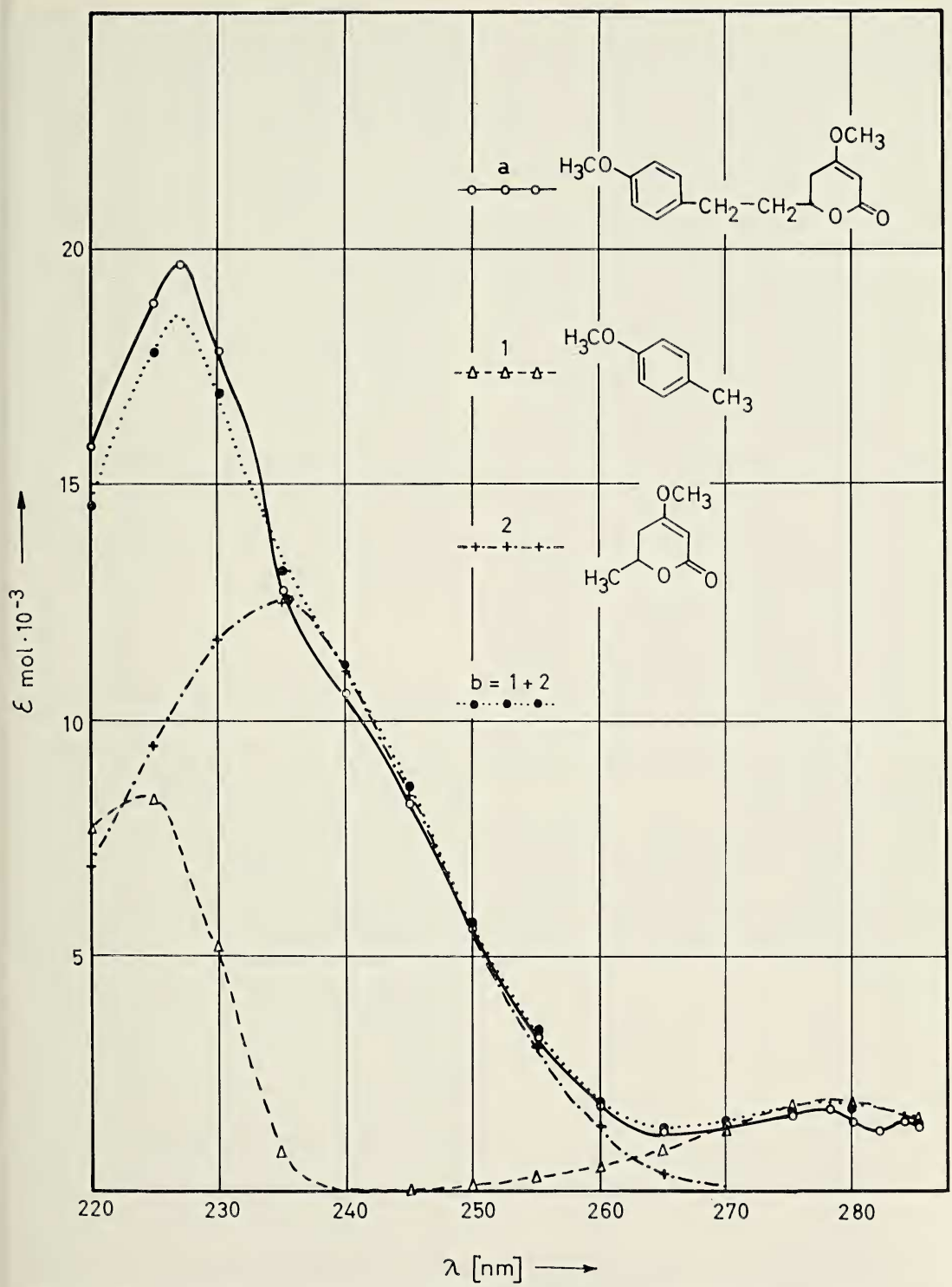


FIG. 16. UV spectra of (*a*) tetrahydroyangonin, and (*b*) the addition spectrum of (*1*) p-cresol methyl ether + (*2*) DH-MTL.

system (Fig. 17). Generally, extension of an enone by an additional conjugated double bond causes a bathochromic shift of 30mμ and an increase of the extinction coefficients in the UV spectra. In our case, the transition from DH-MTL to MTL, the increment is 45mμ and the change in intensity is in the opposite direction, that is, it is a decrease (hypochromic effect). Doubtless this apparent anomaly depends on the fact that the new double bond, which is part of the planar (pseudoaromatic) 6-membered ring, exists in the high energy s-*cis*-conformation. In agreement with this is the striking change in the spectrum when we compare substances with this cyclic chromophore with those which possess a formally identical but open chain chromophoric system. We are dealing here with a transition of a cyclic chromophore (with reference to the C_4–C_5 single bond) which has s-*cis*-conformation to an open chain chromophore with the energetically preferred s-*trans*-conformation (Fig. 17). Changes of conformation of this kind which we have postulated have the described effect on UV absorption. If we substitute a hydrogen atom in the methyl group of MTL, we arrive at a $\triangle_7$-DH-yangonin, which is a type of *kawa* pyrone that is readily accessible by synthesis but has not been found in nature. The UV spectra of these compounds are again additive from the partial chromophores of MTL and substituted toluenes.

The last group of structural variants with respect to degree of hydrogenation is the one in which the lactone ring is connected with the benzene ring by an ethylene bridge. The best known representative of this group is yangonin ($R_1 = OCH_3$, $R_2 = H$) (Fig. 18). Since we

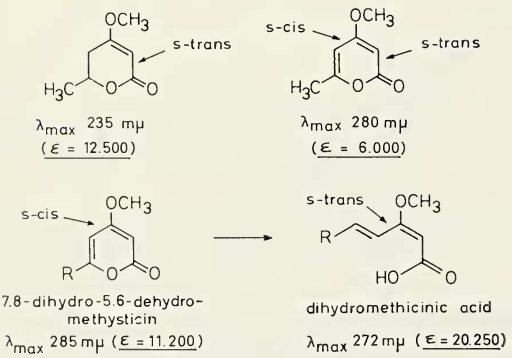


FIG. 17. UV absorption data of the MTL system.

are now dealing with a fully conjugated system, the spectra are no longer simply predictable from partial spectra. The increment ($\Delta\lambda$) of the bathochromic shift is 164mμ for the styryl radical in MTL, and the factor for the enhancement of the intensity of the band is approximately 4.4. The dienolide ring is certainly planar and we may formulate it as a pseudoaromatic system. With regard to position and intensity of the absorption band the pyrones agree with open chain triene acids as long as one takes into consideration the different conformations; however they differ markedly from the sepectra of the analogously substituted stilbenes. Figure 18 shows further that spectra of triene acids and of their methyl esters do not agree with respect to position and intensity of the bands. Possibly the ester consists of mixtures of s-*cis* and s-*trans* conformers with respect to the single bond between the carbonyl carbon and the adjacent carbon. A study of models of these compounds, however, lends no support for possible steric hindrance.

CONCLUSIONS

From the rhizome of *Piper methysticum* or *kawa* a number of constituents have been isolated which are characterized by remarkable pharmacodynamic properties. One may ask why these substances with such remarkable properties have not found any use in modern therapy, for example, as an ataractic or an anticonvulsant. A precise answer to this question is not particularly easy. There are available a large number of chemical compounds, particularly among the readily available synthetic ones, which inhibit the central nervous system. Testing methods for such compounds are well worked out and are plentiful. The probability of finding therapeutically useful substances, therefore, is relatively great. This is particularly so since the sedative-hypnotic-narcotic property of a substance is not structurally specific. In fact, all substances with a given physical-chemical property, for instance, a given partition coefficient (relatively high lipid solubility), represent potential sedative-hypnotic agents. This means that in the case of hypnotic-narcotics one is not very dependent upon models in nature as is the case with other groups of drugs. To this must be

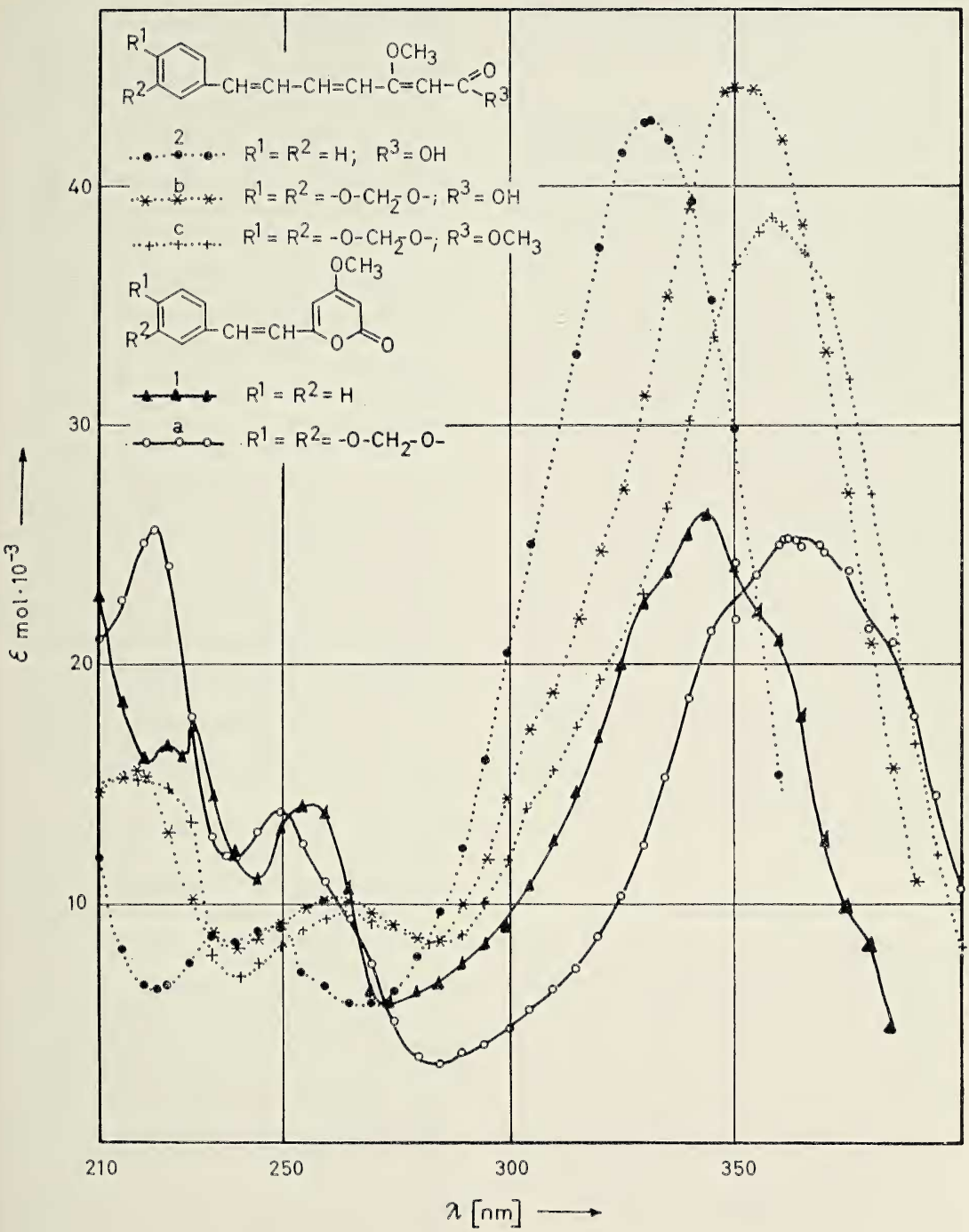


FIG. 18. UV spectra of the yangonin type compounds and their open chain analogs.

added that we expect newly introduced therapeutics to be in some way superior to those which are already in therapeutic use. It appears then that such superiority has not been demonstrated for the *kawa* lactones.*

The further question arises: What is the intrinsic value of such a detailed investigation of the phytochemistry and pharmacology of a single plant? I shall not retreat by responding that every scientific investigation, regardless of the subject or the goal, carries with it its own justification. I should like to express the view that there is scientific justification in learning the chemical composition and the effects of an exotic plant which has played such an important role in the lives of the peoples of Oceania for thousands of years. To this may be added that natural products have always been models and examples for new medicinal agents in biochemical research. Even if this is not the case, as has been pointed out, for the sedative-hypnotic effect, it may perhaps be true for the other effects of *kawa*. Perhaps the *kawa* pyrones will stimulate chemists studying synthetic medicinal products to new research in the fields of epileptic agents, endoanesthetics, or oral antimycotics.

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